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Research Article

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ABSTRACT Human skin fibroblasts synthesize and accumulate glycosaminoglycans (GAG). Recently, we reported that fibroblasts incubated in thyroid hormone-deficient media accumulate more GAG than do cultures incubated in the same media enriched with 0.1 μM triiodothyronine (T_3) (1981. *Endocrinology.* **108:** 2397). The current study characterizes that enhanced accumulation. Confluent cultures were maintained in thyroid hormone-deficient media without or with added T_3, labeled with [^{3}H]acetate and analyzed for total [^{3}H]GAG and [^{3}H]hyaluronic acid content.

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Thus, thyroid hormone inhibits GAG accumulation in a dose-, time-dependent, and reversible manner. This inhibition is apparently due to specific effects on the rate of macromolecular synthesis.

INTRODUCTION

Thyroid hormone modulates many aspects of cellular metabolism (1, 2). In man, hypothyroidism is characterized by a constellation of clinical signs and symptoms, including growth failure and mental retardation (3). In severe, long-standing hypothyroidism, there is a tendency for the deposition of mucinous material in skin, resulting in a characteristic nonpitting edema. This material is composed of acid mucopolysaccharides (glycosaminoglycans, GAG),[1] complex polysaccharides normally found in small amounts (4).

Human skin fibroblasts offer the experimentalist an easily accessible tissue that can be propagated in culture and retain many of the phenotypic expressions typical of differentiated cells. Several biological aspects of the skin fibroblast have been studied in recent years. They are known to retain hormone receptors for triiodothyronine, dexamethasone, insulin, and androgen (5–8). Responses to several hormones including glucocorticoids, prostaglandins, cathecholamines, and insulin have been demonstrated in fibroblasts (9–13).

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[1] *Abbreviations used in this paper*: BS, bovine serum; D-FCS, thyroid hormone-depleted FCS; D-FCS + T_3, D-FCS to which T_3 was added; FCS, fetal calf serum; GAG, glycosaminoglycans; HA, hyaluronic acid; T_3, triiodothyronine; T_4, tetraiodothyronine or thyroxine; Tx-BS, BS from thyroidectomized animals; Tx-BS + T_3, Tx-BS with added T_3; UDP-GlcNAc, uridine disphosphate-*N*-acetyl glucosamine; UDP-GlcUA, uridine diphosphate glucuronic acid.

Little is known about the effect of thyroid hormone in human skin fibroblasts. Current information can be summarized as follows: (*a*) The two metabolically active thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) are transferred from medium to cells. This cellular uptake of hormone is directly proportional to the concentration of free T_4 and T_3 in the medium (14); (*b*) As in other tissues, metabolism of thyroid hormones by fibroblasts involves a conversion of T_4 to T_3 (14); (*c*) Fibroblasts possess high affinity nuclear binding sites for T_3 (5, 15); and (*d*) Observed biologic effects of thyroid hormone include stimulation of growth (16), glucose utilization (14, 17), lactate production (17), and degradation of low density lipoproteins (18).

Recently, we reported that thyroid hormone inhibits the accumulation of GAG in a human skin fibroblast culture incubated in a thyroid hormone-deficient medium (19). The current report describes subsequent studies in fibroblast cultures from several individuals, which were undertaken to determine the kinetics, specificity, reversibility, and dose dependence of this inhibition, as well as to ascertain whether the accumulation of GAG was the result of a changed rate of synthesis or degradation.

METHODS

Materials

[^{3}H]Acetic acid (2,000 mCi/mmol sp act) and uridine diphosphate-[^{14}C]glucuronic acid (UDP-[^{14}C]GlucUA) (343 mCi/mmol sp act), [^{3}H]glucosamine (20,300 mCi/mmol sp act) and L-[^{3}H]leucine (1,110 mCi/mg) were obtained from New England Nuclear, Boston, MA. Streptomyces hyaluronidase was obtained from Miles Laboratories, Inc., Elkhart, IN. All chemicals used were of the highest purity commercially available. Uridine diphosphate sugars were chromatographed on two thin-layer systems to determine purity.

Procedures

Preparation of thyroid hormone-depleted sera. A modification of the method of Samuels et al. (20) was used to remove thyroid hormone from fetal calf serum (FCS). Rexyn 201 or 202 ion-exchange resins (Fisher Chemical Co., Pittsburgh, PA) were washed extensively and were used interchangeably. Resins were incubated with FCS at room temperature on a gyratory shaker. Later experiments utilized bovine serum from a thyroidectomized animal (Tx-BS). The concentrations of total T_4 and T_3 and free (dialyzable) T_3 in various sera are given in Table I. Media supplemented with 10% FCS and BS contained 0.25 and 0.17 nM T_3 respectively, compared with 0.063 and 0.068 nM T_3 for media supplemented with 10% D-FCS and Tx-Bs, respectively. Thus, addition of 0.1 nM T_3 to a medium containing thyroid hormone-depleted serum (D-FCS and Tx-BS) brought the serum total T_3 concentrations to 0.163 and 0.168 nM, and the free T_3 concentrations to 1.39 and 1.01 pM respectively, or to almost physiologic levels. All sera were sterilized by Millipore (0.22 μm) filtration (Millipore, Corp, Bedford, MA) before addition to the culture medium.

TABLE I
Thyroid Hormone Concentration of Sera

Source of serum	Total T_4	Total T_3		Free T_3
	μg/dl	ng/dl	nM	%	pM
FCS	14.5	164	2.52	0.083	2.09
BS	5.1	111	1.71	0.096	1.64
Human serum	8.1	130	2.00	0.100	2.00
D-FCS	<0.2	41	0.63	0.085	0.54
Tx-BS	<0.2	44	0.68	0.060	0.41

Cell culture. Human skin fibroblasts obtained from several subjects by skin punch biopsy, were used between the 6th and 11th serial passages beyond the initial plating. For experiments, cultures were grown to confluence in 60-mm Diam plastic petri dishes using a modified Dulbecco's medium with Earle's salts, 50 μg/ml ascorbic acid, 1 μg/ml ferric nitrate, and enriched with 10% FCS at 37°C in an atmosphere containing 10% CO_2 and a relative humidity of 100%. Glutamine 435 μg/ml and kanamycin 100 μg/ml were added.

Experiments were carried out only after cultures had reached confluence. Cells were then exposed to media containing either normal serum (FCS or BS), thyroid hormone-depleted serum (D-FCS or Tx-BS) or thyroid hormone-depleted serum supplemented with T_3 (D-FCS + T_3 or Tx-BS + T_3) and further incubated for the indicated periods of time.

For [^{3}H]GAG accumulation studies, media were removed 24 h before harvesting and substituted with fresh media containing the same serum and hormone additives with either 10 μCi/ml of [^{3}H]acetate (total 40 μCi/plate) or 5 μCi/ml [^{3}H]glucosamine (total 20 μCi/plate). Various amounts of unlabeled sodium acetate were added to the media in order to examine the influence of the saturable uptake of acetate on the T_3-mediated changes of [^{3}H]GAG accumulation. [^{3}H]Leucine (5 μCi/ml) was added to the culture medium 24 h before harvesting in an experiment that examined the effect of T_3 on protein synthesis. Finally, in the pulse-chase experiment, 60 μCi of [^{3}H]acetate were added in 4 ml of medium per plate and the labeling time was shortened to 12 h. Following three washes of the cell layers with 10 ml serumless medium, 4 ml of the corresponding serum-containing media, devoid of isotope but with 1 mM unlabeled acetate, was added and incubation carried out for various times over 63 h.

Quantification of [^{3}H]GAG accumulation. After labeling, the medium was removed, the cell layer was washed with serumless medium and then disrupted by sonication (sonifier, model 183, Branson Sonic Power Co., Danbury, CT) in 3 ml of 0.1 N NaOH. After removal of an aliquot of the cellular material for protein determination (21), the cell layer and medium were combined, neutralized with HCl, and digested with 1 mg/ml pronase in 100 mM Tris buffer pH 8.0 at 50°C for 16 h, in the presence of 250 μg of both hyaluronic acid (HA) and chondroitin sulfate, which were added as carriers. After cooling on ice, trichloroacetic acid (TCA) was added to a final concentration of 5% and the samples were allowed to precipitate for 1 h. After centrifugation at 10,000 *g* for 10 min, the nonprecipitable material was dialyzed extensively against water at 4°C and then ly-

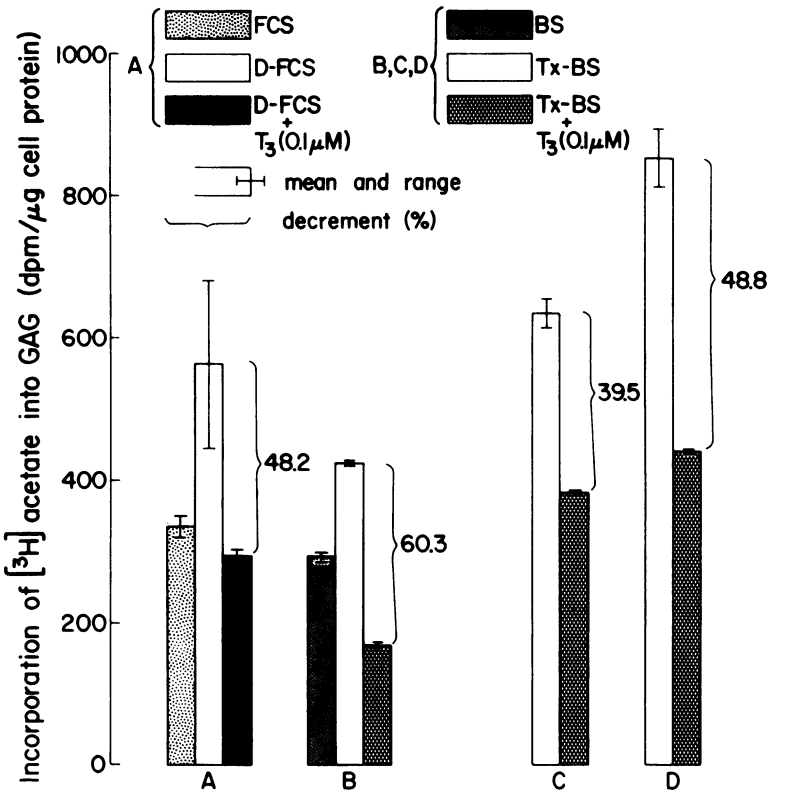

FIGURE 1 Reproducibility of the effect of T_3 on the incorporation of [^{3}H]acetate into GAG. All experiments were conducted on fibroblasts obtained from the same subject, and grown to confluence in media containing 10% FCS. The medium was then substituted as indicated in the figure key, for 3 d before harvest. Experiments A and B were carried out simultaneously, while experiments B, C, and D were conducted at different times over a 5-mo period.

ophilized to dryness. The residue was resuspended in 0.15 M NaCl. A small aliquot was counted in a Packard TriCarb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL) and defined as total [^{3}H]GAG.[2] Results are expressed as mean and range of determinations made on duplicate cultures grown under the same conditions. In some experiments, an aliquot was applied to a G-50 Sephadex column (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) and the material eluted in the void volume was incubated with 10 U of Streptomyces hyaluronidase at 55°C for 20 h. The postdigestion sample was resubjected to Sephadex chromatography using a pyridine acetate buffer pH 5.8. The digested material, eluted after the void volume, was defined as HA. Recovery from chromatography was 95%.

Cell-free hyaluronate synthetase assay. Cultures were grown as described above except that 100-mm Diam plates were used. After 3 d of incubation in media supplemented with D-FCS, D-FCS + T_3 or FCS, cell layers were washed two times with Hanks' balanced salt solution, then scraped off the plates and concentrated by centrifugation. Following two washes with a buffer containing 4 mM dithiothreitol, 200 mM sucrose, 40 mM Hepes, pH 7.1, cells were disrupted by sonication (microtip at 100 W output for 1 s, repeated twice) on ice, and an aliquot was taken for protein determination by the method of Bradford (23). To assay hyaluronate synthetase activity, an aliquot of the sonicate was incubated with 0.32 μmol $MgCl_2$, 16 μmol sucrose, 0.63 μmol UDP-*N*-acetyl glucosamine (UDP-GlcNAc), 3 nmol UDP-glucuronic acid (UDP-GlcUA), 500,000 dpm UDP-[^{14}C]GlcUA, and 3.2 μmol Hepes pH 7.1 in a total volume of 100 μl for 1 h at 37°C. The reaction was terminated by boiling for 5 min followed by centrifugation at 9,000 *g*. Aliquots of the supernatant were subjected to G-50 Sephadex chromatography and eluted with 0.5 M NaCl. Fractions were counted for radioactivity.

RESULTS

Fig. 1 contains data concerning the reproducibility of the observed inhibitory effect of T_3 on the incorporation of [^{3}H]acetate into GAG. The experiments labeled as B, C, and D were carried out at different times, over a 5-mo period, using cultures from the same donor and same batch of Tx-BS. Despite a two-

[2] We have previously shown that >95% of the radioactive material was degraded by hyaluronidase, chondroitinase ABC, and nitrous acid, as expected for GAG (19). In early experiments, postdialyis samples were analyzed for total uronic acid content by the method of Dische (22). Recovery was uniform.

fold variation in the absolute amount of [^{3}H]GAG accumulation, the proportional decline as a result of T_3 addition ranged from 39.5 to 60.3% and did not correlate with the absolute magnitude of [^{3}H]acetate incorporation. The figure also shows that the inhibitory effect of T_3 was reproduced when the hormone was added to either of the two types of hormone-depleted sera, D-FCS or Tx-BS (Fig. 1A and B).

To determine whether the inhibitory effect of T_3 on [^{3}H]acetate incorporation could be due to changes in the saturable uptake of acetate, unlabeled acetate was added in increasing concentrations (0, 100, 500, 2,500 μM) together with the labeled tracer (5 μM). As shown in Fig. 2, despite a 80% decrease in [^{3}H]acetate incorporation caused by the decrease in specific activity, there were no changes in the proportion of inhibition by T_3. The latter ranged from 34.9 to 39.5%. The decrease in the specific activity of [^{3}H]acetate was associated with a decrease in the uptake of [^{3}H]acetate measured in aliquots of the washed cell layer. Uptake was 4.15, 3.40, 2.50, and 1.05% in the presence of 0, 100, 500, and 2,500 μM of unlabeled acetate, respectively. Addition of T_3 did not affect the uptake of the tracer. Furthermore, using fibroblasts from the same donor, T_3 induced a 32.1% inhibition of [^{3}H]glucosamine incorporation into GAG (Fig. 2).

To assess whether the observed effect of T_3 on GAG accumulation is specific rather than a generalized inhibitory action of the hormone, the incorporation of [^{3}H]leucine into proteins was examined under identical experimental conditions. Results are given as mean±SD for duplicate cultures exposed to media supplemented with Tx-BS and TxBS + T_3 (0.1 μM), respectively, for the period of 3 d. [^{3}H]Leucine was added 24 h before harvesting. For fibroblasts exposed to Tx-BS and Tx-BS + T_3, respectively, retention of the isotope in the cell layer was 5.0±0.1% and 4.3±0.2%, and 65.5±4.0% and 82.3±2.2% of the ^{3}H activity in cells was TCA precipitable. The total TCA-precipitable activity in cultures exposed to thyroid hormone-depleted and T_3-supplemented media was not significantly different, namely, 3.78±0.26% and 4.17±0.08% of the total radioactivity added. In both conditions, 87% of the TCA-precipitable radioactivity was recovered from the cell layer. After digestion with pronase, 0.24±0.01% and 0.27±0.2% of the radioactivity was TCA precipitable and only 0.10±0.02% and 0.07±0.01% of the activity remained in the TCA nonprecipitable, nondialyzable GAG-containing material.

The preceding experiments indicated that the inhibitory effect of thyroid hormone on [^{3}H]GAG accumulation could not be accounted for on the basis of gross shifts in precursor pool size. Furthermore, the effect seemed to be somewhat specific in that similar changes in total cellular protein synthesis were not demonstrated. The issue of whether the observed effects of T_3 on [^{3}H]GAG accumulation were a result of changes in the rate of GAG degradation was addressed in a pulse-chase experiment. Cultures were incubated in media supplemented with D-FCS without or with

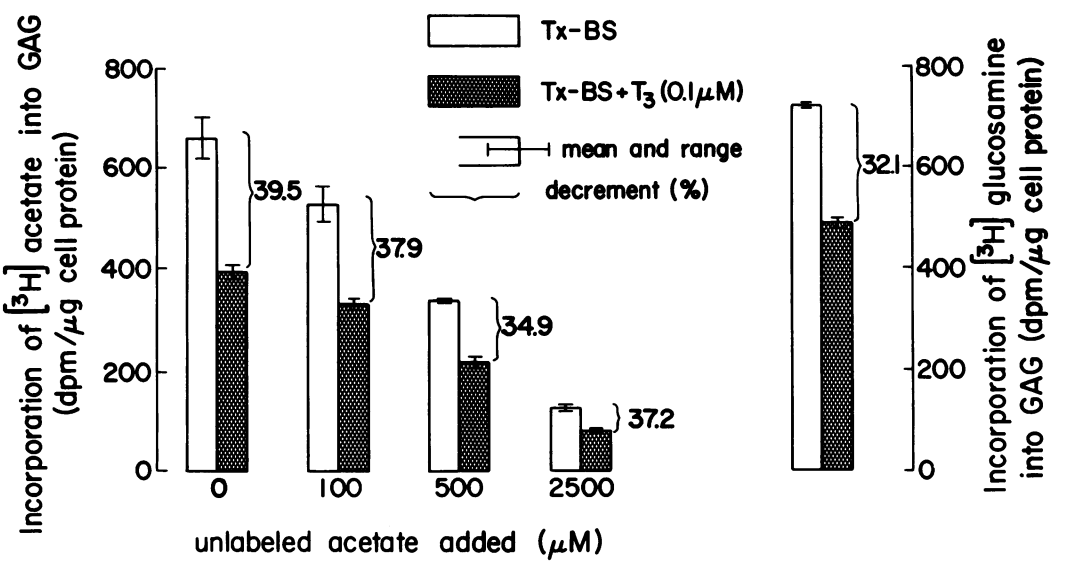

FIGURE 2 Effect of T_3 on the incorporation of [^{3}H]acetate and [^{3}H]glucosamine into GAG. Fibroblasts obtained from the same subject were used in both experiments and were exposed to Tx-BS or Tx-BS + T_3 for 3 d. Addition of unlabeled acetate decreased by 80% the incorporation of [^{3}H]acetate into GAG but did not alter the inhibitory effect of T_3. The inhibitory effect on GAG was observed when both labeled acetate and glycosamine were used as substrates.

TABLE II

Effect of T_3 on [^{3}H]GAG and [^{3}H]HA Degradation in a Pulse-chase Experiment

	Duration of chase (hours)				
	0	12	20	36	63
[^{3}H]GAG $\left(\frac{+T_3}{-T_3}\right)$	0.59	0.64	0.61	0.62	0.65
[^{3}H]HA $\left(\frac{+T_3}{-T_3}\right)$	0.58	0.62	0.56	0.58	0.61
Proportion of [^{3}H]HA in [^{3}H]GAG (%)					
$+T_3$	77.5	77.2	76.8	76.7	77.2
$-T_3$	79.7	81.5	83.8	82.4	82.1

Cultures were incubated either in D-FCS-containing medium ($-T_3$) or in the same medium supplemented with 0.1 μM T_3 ($+T_3$) for 48 h before pulse. The cell layer and media were analyzed for [^{3}H]GAG and [^{3}H]HA. Data are expressed as a ratio $+T_3/-T_3$ for total [^{3}H]GAG or [^{3}H]HA following the chase. For time 0 (before chase), the results are derived from the cell layer analysis alone. The proportion of [^{3}H]HA in [^{3}H]GAG is also given as a percent.

0.1 μM T_3 for 48 h and then labeled for an additional 12 h with [^{3}H]acetate. The chase was carried out for 12, 20, 36, and 63 h. As shown in Table II, total [^{3}H]GAG and [^{3}H]HA content, expressed as ratio in cultures exposed to T_3 to corresponding cultures deprived of the hormone, did not change during the entire period of the chase. This result suggests that the rate of degradation of presynthesized GAG or HA is not affected by T_3. Furthermore, the proportion of [^{3}H]HA in [^{3}H]GAG was similar in fibroblasts exposed to T_3 and deprived of T_3.

Since the rate of GAG degradation could not be implicated in the T_3-induced inhibition of [^{3}H]GAG accumulation, an effort was made to directly assess the rate of synthesis utilizing a cell-free hyaluronate synthetase assay. When cell sonicates from cultures deprived of thyroid hormone were incubated with UDP-[^{14}C]GlcUA, 1,290±62 (n = 3) dpm/mg protein was incorporated into macromolecules compared with 878±27 dpm/mg protein incorporation in sonicates of T_3-repleted cells ($P < 0.005$). 85–90% of the product was digested with Streptomyces hyaluronidase. Thus, cultures maintained in D-FCS + T_3 have 32% less hyaluronate synthetase activity, a proportionally similar T_3 effect to the [^{3}H]acetate incorporation into GAG in a parallel experiment.

To assess whether the effect of thyroid hormone deficiency was reversible, several plates of fibroblasts from one subject were exposed to the D-FCS-supplemented medium for 10 d and then some were replenished with 0.1 μM T_3. At various times, cultures were labeled for 24 h, and then harvested and analyzed for [^{3}H]GAG content. Within 24 h of T_3 addition, a decrease in [^{3}H]GAG accumulation was evident compared with control cultures maintained in the T_3-deprived medium (Fig. 3). This reversal was maximal by 72 h. Because a maximal effect was noted within 72 h of culture in the thyroid hormone-deficient medium, most experiments were conducted after exposure to the hormone-deficient medium, (D-FCS or Tx-BS) for 3 d.

Various amounts of T_3 were added to cultures incubated in D-FCS-supplemented medium (Fig. 4). The greatest amount of [^{3}H]GAG accumulation occurred at the lowest concentration of T_3 (0.06 nM), which represents residual hormone after resin treatment of the FCS. 73% of the maximal inhibitory effect of T_3 was observed at a concentration of 0.16 nM, which was similar to that found in media supple-

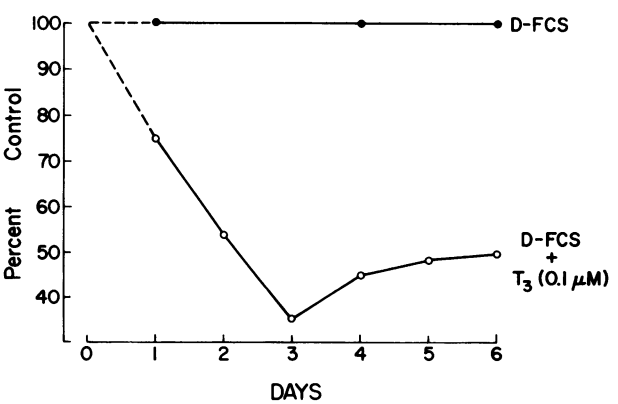

FIGURE 3 Time course of reversibility of the effect of thyroid hormone deficiency on [^{3}H]acetate incorporation into GAG. Confluent cultures were incubated for 10 d in D-FCS. From time 0, 0.1 μM T_3 was added to some cultures (○), while others were continually maintained in D-FCS (●). Data from cultures with T_3 added back are expressed as a percentage of control cultures that remained in D-FCS.

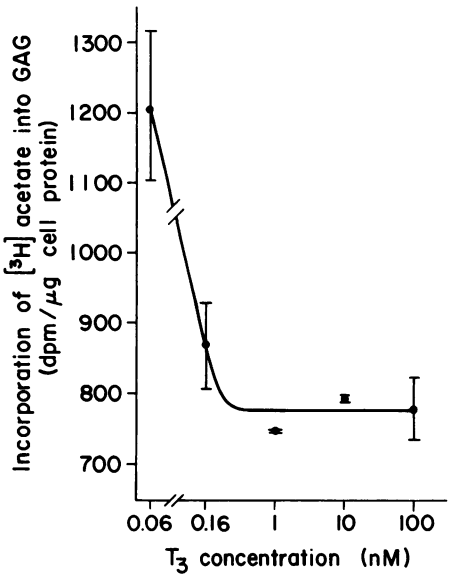

FIGURE 4 Effect of T_3 concentration on [^{3}H]GAG accumulation. Following confluence, cultures were incubated for 3 d in a D-FCS-containing medium in the presence of various concentrations of T_3. The T_3 concentration of 0.06 nM represents the contribution of the T_3 in the D-FCS and, further increments from 0.1 to 100 nM, T_3 added to the medium. Data are expressed as the mean and range of determinations in duplicate cultures.

mented with untreated FCS. The addition of T_3 in concentrations from 1 to 100 nM resulted in no further inhibition. Similarly, the addition of up to 100 nM T_3 to cultures maintained in FCS resulted in no consistent change.

Fig. 5 contains data from an experiment involving skin fibroblasts from four individuals. Following confluence, cultures were exposed for 3 d to media supplemented with 10% Tx-BS without or with 0.1 μM T_3. 24 h before harvesting [^{3}H]acetate was added. Quantitation of [^{3}H]acetate incorporation into macromolecules revealed that cultures incubated in the T_3-supplemented medium accumulated 27.8–53.2% less [^{3}H]GAG than did twin sister cultures deprived of T_3. Despite variation in the absolute amount of [^{3}H]GAG accumulation between subjects, the decline as a result of T_3 addition invariably occurred. Because the protein per cell ratios remained constant in the two incubation conditions, the results are similar whether expressed per microgram cell protein or per cell. The cultures were of similar density when harvested.

DISCUSSION

GAG metabolism has been studied in in vitro systems as a function of development (24), hormone action (25), cell density and transformation (26), and has been correlated with such cellular metabolic events as cyclic AMP accumulation (27). The biological role of these complex sugars, however, has yet to be fully understood. There is evidence that they are important in cell attachment to substrate, communication with other cells, and, perhaps, insulate cells from chemical perturbations in the surrounding milieu (28).

Human skin fibroblasts are known to elaborate large amounts of GAG, most of which is HA, with considerably smaller amounts of chondroitin sulfate, heparan sulfate, and dermatan sulfate (26). A large proportion of HA synthesized in the cells is extruded into the medium (26, 29). Many aspects of HA synthesis are not well understood. Chain elongation, by sequential transfer of GlcUA and GlcNAc from their UDP donors, is independent of protein synthesis. Human skin fibroblast hyaluronidase has not been identified (30). This is not true of some animal fibroblast systems (31).

Our interest in the effect of thyroid hormone on GAG was prompted by in vivo observations (3, 4) and by our early work showing an inhibitory effect of T_3 on [^{3}H]GAG accumulation in fibroblasts from a single human donor (19). The present work contains data which indicate that earlier observations of inhibition of [^{3}H]acetate incorporation into GAG by T_3 is most likely due to a decrease in GAG synthesis. Indeed, we have shown that changes in the specific activity of added acetate that cause an 80% inhibition in the cell uptake of [^{3}H]acetate and its incorporation into GAG, does not obliterate the effect of T_3. A similar inhibiting effect of T_3 was obtained using [^{3}H]glucosamine as a

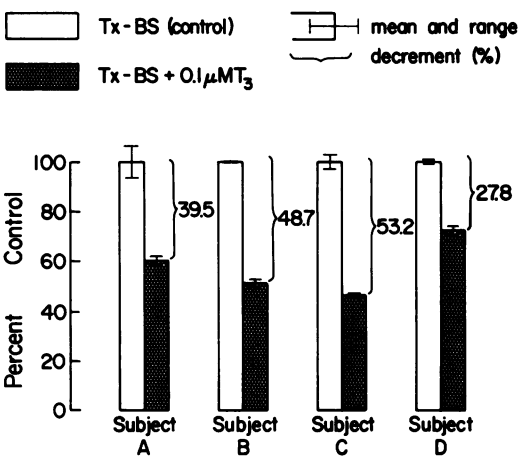

FIGURE 5 Effect of thyroid hormone on [^{3}H]acetate incorporation into GAG in fibroblast cultures from four subjects. Cultures were grown to confluence in medium supplemented with FCS (Methods). Cultures were then shifted to a Tx-Bs-containing medium, without or with 0.1 μM T_3 for an additional 3 d of culture. The T_3-induced suppression [^{3}H]acetate incorporation into GAG is indicated in percent±range of the untreated controls.

substrate. Under identical experimental conditions, T_3 had no effect on total protein synthesis as assessed by the incorporation of [^{3}H]leucine into TCA-precipitable material. Results of the pulse-chase experiment suggest that the observed effects of T_3 cannot be explained on the basis of an enhanced rate of GAG degradation. Further, the data suggest that no significant amount of degradation of hyaluronidase-sensitive material has occurred over the course of the incubation, in keeping with a previous failure to demonstrate hyaluronidase activity in human skin fibroblasts (30). These observations, together with the results obtained with the cell-free hyaluronate synthetase assay, strongly suggest an inhibitory effect of thyroid hormone on HA synthesis. It is unclear whether T_3 affects chain elongation or *de novo* synthesis. Further, it is not possible to determine whether the decreased synthetase activity is due to decreased or altered enzymes. The results do imply that some factor in polysaccharide synthesis is altered.

The inhibitory effect of thyroid hormone on GAG synthesis was reproducible using cultures from the same subject, and was present in fibroblast cultures from four other normal individuals. The dose range of T_3 which inhibits GAG synthesis was similar to that stimulating cytoplasmic growth hormone mRNA accumulation and growth hormone synthesis in rat pituitary cells (32, 33). Changes were observed between the subphysiological levels of T_3 (0.4 and 0.5 pM of free T_3) and the physiological range of concentrations (1.6–2.1 pM of free T_3). The effect is due to T_3 rather than to other serum components, since it was observed in the presence of normal FCS depleted of thyroid hormone by resin treatment as well as with untreated BS obtained from a thyroidectomized animal. The relatively rapid reversibility of the effect of hormone deficiency and lack of effect on total protein synthesis suggest that, under the culture conditions chosen, thyroid hormone absence per se does not have generalized "toxic effects". Because hormone perturbations were made only after confluence and contact inhibition was achieved in normal growth conditions, the effects seen are those on a resting, nondividing population of cells. This strategy allows the assessment of nongrowth-related hormone responses. Since GAG accumulation per cell is known to vary with the phase of growth to confluence (26), uniformity in culture density is essential if conclusions are to be drawn regarding a primary hormone effect on GAG.

Estrogen and testosterone, as well as thyroid hormone, have been shown to have a variety of effects on GAG accumulation in different tissues (4, 34–37). Thyroid hormone appears to enhance the incorporation of [^{35}S]sulfate into chondroitin sulfate in chick embryo costal cartilage (25). Schiller et al. (37), demonstrated that thyroid hormone depletion resulted in an increased HA content in the skin of the rat. The findings in vitro are consistent with previously reported effects in vivo.

The finding of thyroid hormone inhibition of GAG synthesis prompts some speculations as to what effects, if any, the hormone may have upon other macromolecular metabolism. Preliminary work in this laboratory suggests that collagen accumulation may also be stimulated under T_3-deprived conditions (unpublished observations). These effects are perhaps related to common pool shifts or possibly to a coordinate shift in cellular energy expenditure in the compromised cell. Fibroblasts obtained from patients with Marfan's syndrome are also known to elaborate greatly increased amounts of HA when compared with normal controls (29). The increase appears to be due to enhanced rates of synthesis (38) analogous to the situation depicted here. The notion that a common mechanism for the two different entities exists is an intriguing one.

The present data suggest that thyroid hormone regulates GAG synthesis and accumulation in a dose- and time-dependent manner in vitro consistent with previously reported thyroid-sensitive metabolic events. Casting this hormone in the role of an inhibitor is somewhat contrary to the pervasive attitude that it is a stimulator. Recent reports that erythrocyte membrane Ca^{++}-ATPase activity from mature rats is inhibited by T_3 (39) and that thyroid hormone both enhances and inhibits pretranslational events (40), together with the results reported here, shed new light upon the complexities of hormone-regulated gene expression in mammalian cells.

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